REMARKS

Reconsideration and allowance in view of the foregoing claim amendments and the following remarks are respectfully requested.

Claims 1, 2, 3, 5, 7, 13, and 17 have been amended. Claims 1, 7, 13, and 17 have been amended to remove objectionable language "or a portion". Claim 2 has been amended to correct a spelling error. Claims 3 and 5 have been amended to remove objectionable language and to more clearly define the invention as pertaining to a natural or synthetic variant of SEQ ID NO:1 or SEQ ID NO:2. It is well known in the art that variant strains of *Y. pestis* occur, and, enablement is provided in the application as filed to prepare a DNA fragment according to the present invention containing natural and introduced changes in the DNA sequence which would still encode an F1-V fusion protein. Support for the claim is found in the application as filed, for example on page 10, lines 18-27 and continuing on page 11, lines 1-15, on page 23, lines 5-12, and in claim 21 as originally filed. Entry and consideration of the claims as amended are respectfully requested.

Claims 31 and 32 have been added. The new claims are drawn to a DNA fragment comprising F1 capsular antigen from *Yersinia* pestis fused to a V antigen from other *Yersinia* species which have a V antigen homologous to that of *Yersinia* Pestis. Claim 32 defines the other *Yersinia* species as *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*.

Support for the new claims is found in the specification and claims as filed, specifically on page 2, lines 16-19, page 7, lines 19-24, and claim 24. Entry and consideration of the new claims are respectfully requested.

Claims 1-17, 30-32 are pending in the subject application.

Claim 4 stands objected to under 37 C.F.R. 1.75(c) as allegedly of improper dependent form. Claim 4 has been cancelled without prejudice. Withdrawal of the rejection is respectfully requested.

Claims 1-17 and 30 stand rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite for use of the term "portion". The claims have been amended to remove objectionable language. Withdrawal of the rejection is respectfully requested.

Claims 11 and 15 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make or use the invention with regard to pF1V, and *E. coli* BLR. This rejection is traversed in view of the following.

E. coli BLR is commercially available from Novagen, Madison, Wisconsin as disclosed in the application. on page 16, lines 8 and 9, for example. The characteristics and description of this *E. coli* strain are known and provided by Novagen.

Claim 11 is drawn to the recombinant construct pF1V. pF1V is composed of the original expression vector pET19b, commercially available from Novagen, Madison, Wisconsin, into which was inserted an F1-V DNA fragment was ligated. The F1 gene was obtained by amplifying using the standard polymerase chain reaction techniques from the plasmid pYPR1 (obtained from Rocky Mountain Labs, Hamilton, Montana) using primers specified in SEQ ID NO:3, SEQ ID NO:4. The V antigen was obtained using standard PCR techniques using primers specified as SEQ ID NO:5, and SEQ ID NO:6. These fragments were ligated into the commercially available expression vector pET19b as described in the specification on pages 15 and 16, and shown in schematic representation in Figure 1, using standard, readily reproduced procedures. The resulting sequence of the F1-V fragment inserted and expressed by pF1-V is additionally described on page 15, line 9-19 and provided in the Sequence Listing originally filed with the application.

Applicants submit that a person with ordinary skill in the field of molecular biology, using the disclosed description of pF1V, should be able to reproduce the plasmid. Since pF1V is enabled due to the reproducible description of the plasmid, and the *E. coli* strain is enabled by its commercial availability, the claims are enabled, and withdrawal of the rejection is respectfully requested.

Claims 1-10, 12-17 and 30 stand rejected under 35 U.S.C. §112, first paragraph as allegedly nonenabling for DNA

encoding mutants, or truncations or SEQ ID NO:1 or 2. The claims as amended are drawn to the entire sequence or F1 or V, and the objectional language "a portion thereof" has been removed. Natural or synthetic variants claimed are expected to still encode both the immunologically identifiable F1 and V antigens. It would not require undue experimentation by a person with ordinary skill in the art to produce synthetic variants, which differ in nucleotide sequence but still encode the desired antigen, for example, due to codon redundancy, and retain its protective properties. In fact, when Applicants sequenced F1-V fusion DNA fragment, two nucleotides were different between the F1-V fusion fragment and the previously published V antigen, as disclosed on page 16, lines 14-19. One of the differences resulted in no change in the amino acid at that site, and the other one resulted in a change from alanine to threonine.

Due to the degree of knowledge of F1 and V sequences providing predictability in the art, and due to a clear description of the required characteristics of the final DNA fragment envisioned, i.e., still encoding the complete F1-V fusion, which provide adequate guidance with regard to producing an F1-V fusion, production of a variant as claimed would not require undue experimentation to practice the invention. Reconsideration and withdrawal of the rejection are respectfully requested.

Claims 1-6 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by Price *et al.* (*J. Bacteriology*

171:5646-5653, 1989). The claims as amended are drawn to a complete sequence of F1 and V and are believed to render this rejection moot. Withdrawal of the rejection is respectfully requested.

Claims 1-17, and 30 stand rejected under 35 U.S.C. §103(a) as allegedly obvious over WO95/18231 (Titball *et al.* -'31) and further in view of: WO 95/24475 (Titball *et al.*- '75); or Leary *et al. Infection and Immunity* **63**: 2854-2858, 1995). This rejection is traversed since none of the references cited teach or suggest fusion of F1 with V antigen.

It was first demonstrated in 1952 that F1 is a protective immunogen (Baker *et al. J. Immuno.* **68**:131-145, 1952) and in 1963 that V antigen is a protective immunogen (Lawton *et al. J. Immunol.* **91**:179-184, 1963). Subsequent workers have only confirmed these findings, most recently using F1 (Simpson *et al. Am. J. Trop. Med. Hyg.* **43**:389-396, 1990) and V (Leary *et al. Infect. and Immun.* **63**:2854-2858, 1995, cited by Examiner) derived from recombinant DNA technology.

Titball *et al.*-'31 discloses F1 protein and its sequence. The recombinant DNA encoding fusion proteins disclosed by Titball '31 on pages 5 and 6 as mentioned by Examiner refer to fusions to β-galactosidase and other signal sequences to potentially facilitate secretion of the F1 or to facilitate cloning. Similarly, Titball '75 generate fusions of V to proteins to facilitate purification of V, such as

maltose binding protein and glutathione-S-transferase (page 8). Leary *et al.* reported on the use of a fusion protein of V and glutathione-S-transferase to enable efficient purification of the V protein. No suggestion is present in any of the references cited by Examiner to fuse F1 and V.

Most importantly, there is no motivation in the art to produce a fusion protein comprising the F1 antigen and the V antigen. F1 is encoded on a 100 kb plasmid, and is found on the surface of the bacterium capsule (please see the specification, page 2, lines 6-15). The V antigen is encoded by a different plasmid of 75 kb, and is a secreted protein which regulates the transcription of other virulence genes (please see specification, page 2, lines 16-25). F1 and V are not found connected in any way in the bacteria itself, nor in the host during infection.

Since the F1 and V antigens are not found connected in their natural configuration, and it is well known that fusion of one protein with another may result in instability and degradation of the resultant fusion protein, perhaps due to improper folding of the antigens when they are fused (E. Amann. The Utility of Fusion Proteins in the Development of Vaccines. In: Recombinant DNA Vaccines (R.E. Isaacson, ed. Marcel Decket, N.Y., 1992) resulting in possible loss of immunogenicity, there is no motivation or expectation that a fusion protein comprising two antigens would be stable and immunogenic

In fact, initial attempts by Applicants to fuse the F1 antigen with a portion of the V antigen (amino acids 168-275) known to be immunogenic resulted in a fusion protein which was unable to effectively immunize mice against a subcutaneous challenge with $F1^-$ strain, i.e. the V fragment, a previously immunogenic peptide, was no longer able to elicit a protective response in the animal once fused to another protein. Please see the specification on page 22, lines 8-20 for a discussion of this F1-trucated V fusion protein.

In view of the lack of motivation in the art, the absence of expectation of success, the negative teaching in the art with regard to fusing proteins required for immunogenecity, and due to the lack of motivation or suggestion provided by any of the references, alone or in combination, to fuse F1 with V, Applicants submit that the fusion of F1 and V is novel and unobvious. Withdrawal of the rejection is respectfully requested.

All objections and rejections have been addressed. This application is believed to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

By SAPratt Reg No. 39,441
for Charles H. Harris
Reg. No. 34,616

U.S. A. MRMC
504 Scott Street
Fort Detrick, MD 21702-5012
ATTN: MCMR SGRD-JA (Charles H. Harris - Patent Atty)

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to the

Commissioner of Patents
Washington, D.C. 20231

on April 10, 1998.

By SAPratt
Sana A. Pratt
Reg. No. 39,441